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Contrasting elevational diversity patterns for soil bacteria between two ecosystems divided by the treeline

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Above- and below-ground organisms are closely linked, but how elevational distribution pattern of soil microbes shifting across the treeline still remains unknown. Sampling of 140 plots with transect, we herein investigated soil bacterial distribution pattern from a temperate forest up to a subalpine meadow along an elevational gradient using Illumina sequencing. Our results revealed distinct elevational patterns of bacterial diversity above and below the treeline in responding to changes in soil conditions: a hollow elevational pattern in the forest (correlated with soil temperature, pH, and C∶N ratio) and a significantly decreasing pattern in the meadow (correlated with soil pH, and available phosphorus). The bacterial community structure was also distinct between the forest and meadow, relating to soil pH in the forest and soil temperature in the meadow. Soil bacteria did not follow the distribution pattern of herb diversity, but bacterial community structure could be predicted by herb community composition. These results suggest that plant communities have an important influence on soil characteristics, and thus change the elevational distribution of soil bacteria. Our findings are useful for future assessments of climate change impacts on microbial community.

elevational gradient, treeline, soil bacteria, Illumina MiSeq, diversity pattern, community structure

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INTRODUCTION

Dramatic turnover in climate and biota over short geographic distances is a typical characteristic of elevational gradients (Bryant et al., 2008; Körner, 2007). Understanding patterns of biological diversity along such gradients as well as driving forces behind them is crucial for predicting how biodiversity and ecosystems respond to global climate change (Lomolino, 2001; Loreau et al., 2001). Unlike well-known monotonically decreasing (Stevens, 1992) or unimodal patterns (Guo et al., 2013; McCain, 2005; Rahbek, 1995) of macroorganisms, elevational diversity patterns of microorganisms remain poorly understood and tend to be more complicated.

Since Bryant et al. pioneered the study of elevational richness patterns in soil bacteria, investigations of microbial community distribution have been the spotlight of biological research (Bryant et al., 2008). These studies, however, have revealed different and sometimes conflicting patterns including no apparent patterns (Fierer et al., 2011; Shen et al., 2013), decline patterns (Bryant et al., 2008; Wang et al., 2015a), hump-backed patterns (Miyamoto et al., 2014; Singh et al., 2012) and hollow patterns (Wang et al., 2012a, 2015b) of microorganisms diversity along elevational gra-

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dients. Some scientists suggested that microbes have small body size and are sensitive to local soil environmental conditions, thus would not form significant elevational trends (Fierer et al., 2011; Shen et al., 2014). Whereas others showed that climate change along elevational gradients (Bahram et al., 2012; Singh et al., 2014) and the mid-domain effect (Miyamoto et al., 2014) might in fact have a profound impact on the distribution of microbes. Sampling heterogeneity and scale effects may also influence our perception of patterns (Rahbek, 2005). Moreover, the conflicting results mentioned above may be related to a relatively low and varying sequencing depth. For instance, studies based on 4,000 sequences per sample at Changbai Mountain (Shen et al., 2013) and 1,300 sequences per sample in the eastern Andes (Fierer et al., 2011) showed no elevational patterns in soil bacterial diversity, whereas a recent study of 20,000 sequences using Illumina sequencing showed a clear decreasing pattern (Zhang et al., 2015). Deep-depth sequencing using new technology is therefore needed to re-evaluate the elevational patterns of microorganisms at a higher level of sequences (Caporaso et al., 2012; Logares et al., 2014; Jiang and Hu, 2014).

In addition, many studies have attempted to correlate elevational diversity patterns of microorganisms with the already observed patterns of macroorganisms (Bryant et al., 2008; Shen et al., 2014; Zhang et al., 2015). While studies found that microbes do not follow the elevational diversity patterns of plants (Fierer et al., 2011; Shen et al., 2014). Many studies also have failed to find any significant relationship between the alpha diversity of plants and microbes at the continental scale (Millard and Singh, 2010; Prober et al., 2015). However, plants are considered to be closely associated with microorganisms (Hooper et al., 2000; van der Heijden et al., 2008). Plant diversity can influence the diversity of soil bacteria via rhizodeposits (Ding et al., 2015) or by changing soil conditions (De Deyn and Van der Putten, 2005; Wardle et al., 2004), and distinct plant communities generally are expected to have different microbial assemblages in their soil (Prober et al., 2015). Therefore, variable patterns in soil environmental factors under different types of vegetation may have influence on microbial distribution.

Treeline is considered to be the upper limit of elevational distribution of trees, above which forests are replaced by montane shrubland or grassland that are adapted to low temperature and poor nutrients (Harsch et al., 2009; Körner, 1998; McNown and Sullivan, 2013). Along with the abrupt changes of vegetation, the climate condition and soil characteristic go through great changes. What would these changes act on underground microbial communities? However, little is known about the diversity pattern of soil microbes along the whole elevational gradient, especially how the microbial diversity pattern varies at the treeline (Ding et al., 2015; Thébault et al., 2014). Previous studies have mainly focused on samples from lower elevations, including only one or two samples beyond the treeline (Shen et al., 2013; Singh et al., 2012). For example, a study at Changbai Mountain revealed that bacterial communities in the tundra were not unique (Shen et al., 2013). To better understand the distribution of soil microbes changing with plant communities and the climate change, a higher spatial resolution study may be crucial to detect variations of soil microbial distribution and underlying mechanisms across the treeline along an elevational gradient.

In this study, we investigated the soil bacterial distribution pattern from the oak-dominated temperate forest up to the meadow on an elevational transect using Illumina MiSeq Sequencer at Dongling Mountain, Beijing, China. We aimed to test: (i) whether the soil bacteria diversity show an elevational pattern; (ii) if the distribution pattern and underlying mechanism vary at the treeline and (iii) the relationship between soil bacterial distribution and plant communities.

RESULTS

Plant species richness and soil characteristics across elevations

Richness of herbaceous plant was found to be sensitive to the elevation which was lowest around 1,200 m, increased with the elevation and peaked at the treeline. Above the treeline, the richness of herbaceous plant followed an opposite change, decreasing with elevation in the meadow (Figure S1 in Supporting Information). Elevational patterns of soil variables were contrasting between the forest and meadow (Figure 1). Soil variables except soil texture and pH showed significant correlations with the elevation in the meadow ($P<0.05$, Figure 1). Variations of soil variables in the forest were much smaller than in the meadow but were also correlated with elevations except soil pH, silt% and TN.

Bacterial community composition

Across all the 140 soil samples, we obtained total 3,225,694 high-quality sequences with a range from 11,186 to 34,869 in each sample. In total, 9,132 OTUs were identified, among which 10 OTUs were unique to the meadow and 916 OTUs merely occurred in the forest. A total of 39 phyla were identified. The three most dominant phyla (*Proteobacteria*, *Actinobacteria,* and *Acidobacteria*) accounted for over 70% of the total sequences, followed by *Planctomycetes*, *Chloroflexi*, *Bacterodetes*, and *Gemmatimonadetes* (Figure 2).

Elevational patterns of bacterial diversity

Soil bacterial diversity, as estimated by phylotype richness (OTUs) and phylogenetic diversity (Faith's PD), showed different patterns along elevational gradients in the meadow and forest. The OTUs and PD index followed hollow patterns along the elevational gradient in the forest, with a rela-

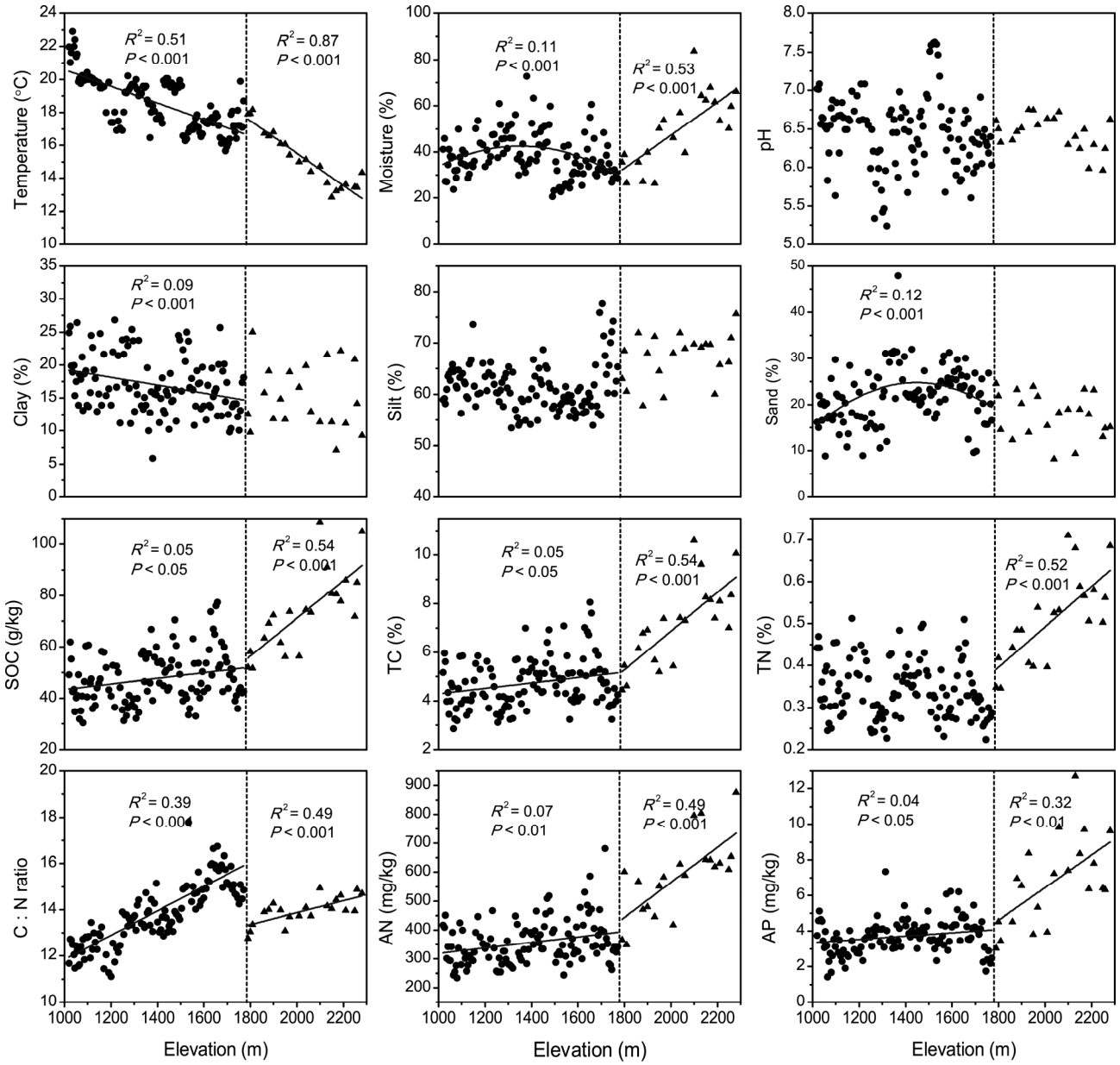

Figure 1 Variation of soil physicochemical variables along the elevational gradients. The dotted line represents the occurrence of treeline. The solid lines indicate the trends (P<0.05). ST, soil temperature; SM, soil moisture; C/N, ratio of total carbon and total nitrogen; TP, total phosphorus; AP, available phosphorus; clay, silt, sand, soil texture; SOC, soil organ carbon.

tively low diversity at elevations ranging from approximately 1,350–1,600 m (Figure 3A). In contrast, soil bacterial diversity significantly declined along the elevational gradient in the meadow above the treeline (Figure 3B).

The bacterial species richness did not show any significant association with herb species richness in either the forest or meadow (Figure S2 in Supporting Information). Multiple regression analysis indicated significant impact of environmental factors on the bacterial diversity patterns (Table 1). Bacterial phylotype richness (OTUs) and phylogenetic diversity (PD) were best explained by environmental factors such as soil temperature, pH, and C∶N ratio in the forest, as well as soil pH and available phosphorus in the meadow (Table 1).

Relationship of biotic and abiotic factors with bacterial community structure

The relative abundance of the dominant phyla (*Proteobacteria*, *Acidobacteria*, *Planctomycetes*, *Chloroflexi*, and *Gemmatimonadetes*) showed significant difference between the forest and meadow (Figure 2). For example, the most dominant phylum, *Proteobacteria*, accounted for 37.4% and 39.4% in the forest and meadow, respectively, while the abundance of *Acidobacteria* was 16.5% and 13.1%, respec-

Table 1 Relationship between bacterial phylotype richness (OTUs), phylogenetic diversity (PD) and potential explanatory variables modelled by multiple ordinary least squares (OLS) regression[a)]

		R^2	Explanatory variables and β-weights[§]		
Forest	OTUs	0.167	ST (0.215)**	pH (–0.171)*	
	PD	0.197	ST (0.220)*	pH (–0.200)*	C∶N (–0.185)*
Meadow	OTUs	0.611	pH (0.632)***	AP (–0.481)**	
	PD	0.437	pH (0.563)**	AP (–0.385)*	

a) ST, soil temperature; C∶N, ratio of total carbon and total nitrogen; AP, available phosphorus. §, Standardized partial regression coefficients; ***, P<0.001; **, P<0.01; *, P<0.05.

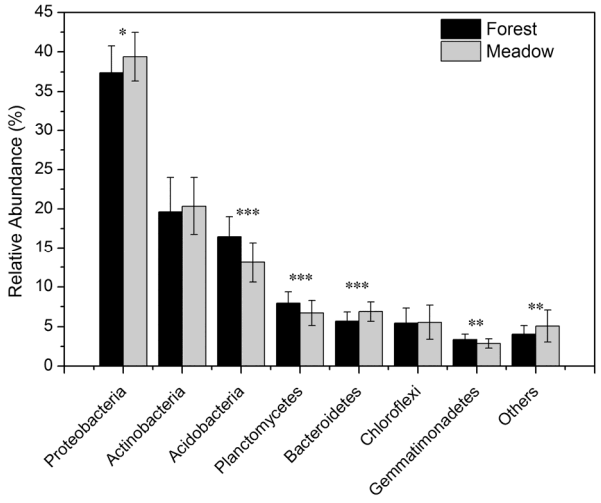

Figure 2 Relative abundance of dominant bacterial phyla between forest and meadow (*t*-test; *, P<0.05; **, P<0.01; ***, P<0.001). All data are presented as mean±SD.

tively. Furthermore, at the OTUs level, PCoA analysis based on Bray-Curtis dissimilarity matrix showed that the bacterial community structure between forest and meadow were well separated from each other in the ordination space (Adonis $F_{1,138}$=9.116, R^2=0.062, P=0.001; Figure 4).

When examining the bacterial communities within both the forest and meadow using PCoA analysis, the bacterial community structure in both areas showed significantly variation along different environmental gradients (Figure 5), with the first axis of the PCoA analysis accounting for 11.9% and 19.1% of variation in the forest and meadow, respectively. According to the partial Mantel test, the environmental distance and dissimilarity of the herb plant community showed significant correlations with the pair-wise dissimilarity matrix of the bacterial community even after other distance matrices were controlled (Table S1 in Supporting Information). The pair-wise dissimilarity of bacterial community and herbaceous plant community had significant positive correlations after controlling for other distances (forest: R=0.27, P=0.0001; meadow: R=0.53, P=0.0001), despite a limited correlation to high dissimilarity in plant composition among some sites in the forest (Figure 6). In total, however, the pair-wise dissimilarity of the bacterial community was lower than that of the plant community.

Further analysis using multiple regressions on distance matrices showed that significantly environmental factors explained 23% and 67% of all variations in the forest and meadow bacterial communities, respectively (Table 2). Soil pH was the main controlling factor in the forest but not in the meadow, where soil temperature was more important (Table 2). PCoA analysis also showed that there was a general clustering of samples in responding to pH gradients in the forest (Adonis $F_{2,116}$=5.688, R^2=0.089, P=0.001; Figure 5A). There was also a significant clustering with different elevational gradients in meadow (Adonis $F_{6,14}$=1.785, R^2=0.434, P=0.001; Figure 5B). Besides soil pH and temperature, soil AP, C∶N, and clay % also played important role in structuring of bacterial community. The spatial distance did not show significant impacts on the pair-wise composition of bacteria neither in partial mantel test nor in the best environmental models (Table S1 in Supporting Information).

DISCUSSION

Different elevational patterns of bacterial diversity above and below the treeline

Although previous studies revealed different elevational trends for bacterial diversity (Bryant et al., 2008; Fierer et al., 2011; Wang et al., 2011), our study showed that the bacterial diversity was a hollow distribution pattern in the forest, and then became monotonically decreasing along the elevation in the meadow above the treeline. The hollow

Table 2 Multiple regressions of distance matrices (MRM) analysis of bacterial community composition against environmental factors for forest and meadow[a)]

	Variables	R^2	a
Forest	pH	0.23	0.014***
	AP		0.005**
	C∶N		0.004*
Meadow	ST	0.67	0.023***
	AP		0.012***
	pH		0.008**
	Clay		0.007**

a) AP, available phosphorus; C∶N, ratio of total carbon and total nitrogen; ST, soil temperature. a, partial regression coefficients; ***, P<0.001; **, P<0.01; *, P<0.05. The most important factors are indicated in bold.

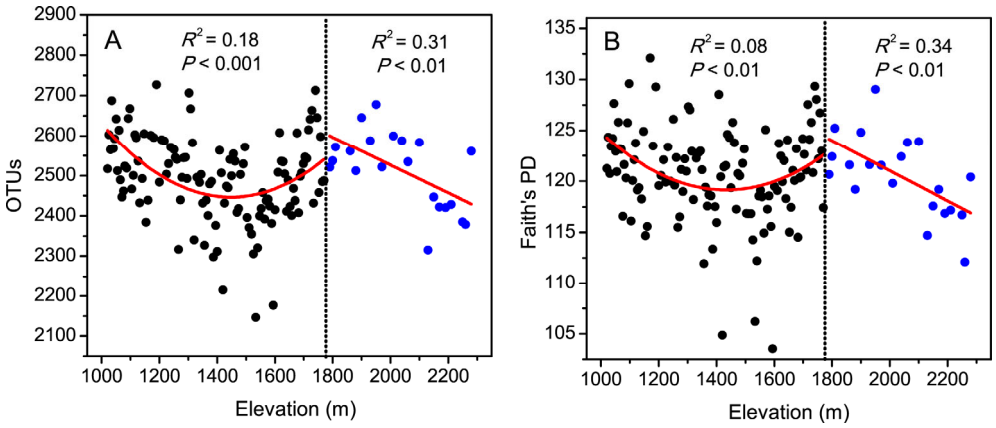

Figure 3 (Color online) Elevational patterns of bacterial phylotype richness (A) and phylogenetic diversity (B). The left side of the dotted line represents the forest gradient (1,020–1,770 m), the right side represents the meadow gradient (1,790–2,280 m).

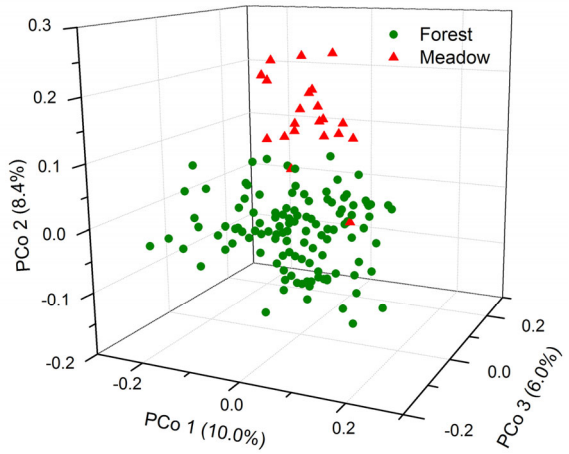

Figure 4 (Color online) PCoA analysis of bacterial communities between the forest and meadow based on Bray-Curtis dissimilarity matrices. The values for PCo 1, 2 and 3 are variation percentages.

pattern of bacterial diversity in forest is not unique although it has been rarely observed in the macroorganism distribution (Rahbek, 2005), which commonly form a middle mountain effect (Bachman et al., 2004). A study of the soil bacterial community on Mt. Halla also showed a hollow pattern along an elevational gradient ranging from 150–1,700 m (Singh et al., 2012) and another study focusing on the aquatic bacteria also demonstrated a hollow pattern in responding to changes in temperature and dissolved organic matter (Wang et al., 2012a). Similarly, Wang et al. revealed a hollow elevational diversity pattern of fungi in Tibet related to soil pH (Wang et al., 2015b). The monotonically decreasing diversity pattern in the grassland was consistent with a recent study in tundra where soil carbon and nitrogen were considered as the limiting factors (Shen et al., 2015).

The bacterial phylotype richness (OTUs) was not correlated with herbaceous plant species richness along the elevational gradient in both the forest and meadow. These results were consistent with previous studies in which microbes did not follow the elevational distribution of macroorganisms (Fierer et al., 2011; Wang et al., 2011) and were poorly related to the alpha diversity of plant on a continental scale (Fierer and Jackson, 2006; Prober et al., 2015). Together with previous studies, our study showed that the underlying mechanisms driving the distribution of microorganisms and macroorganisms were different (Fierer et al., 2011; Shen et al., 2014; Wang et al., 2011). Soil microbes may be more sensitive to soil environmental conditions (Shen et al., 2014), particularly pH values (Baker et al., 2009; Nicol et al., 2008; Shen et al., 2013). In our study, soil temperature, pH, soil available phosphorus, and C∶N ratio were the main factors affecting bacterial diversity even with a narrow pH range.

The distinct elevational patterns between the forest and meadow might relate to the distinct soil environmental conditions regulated by aboveground vegetation (Cline and Zak, 2015; Urbanová et al., 2015; Wardle et al., 2004). Although the diversity of soil bacteria and plant was uncoupled, soil variables showed contrasting patterns between the forest and meadow. In forest, soil temperature and pH determined the bacterial diversity pattern. However, available phosphorus rather than soil temperature played an important role in driving the decreasing bacterial diversity pattern in meadow. Taken together, distinct elevational diversity patterns of microorganisms may occur in response to their environmental conditions influenced by different vegetation.

Linking bacterial communities to vegetation and soil characteristics

Above- and below-ground organisms are closely linked (Wardle et al., 2004) either directly through ecological linkages (Van Der Heijden et al., 2008; Zobel and Öpik, 2014) or indirectly through soil processes (Landesman et al., 2014). Above-ground plants can affect soil microbial community composition via rhizodeposits (Ding et al., 2015) or by modifying the soil environmental conditions

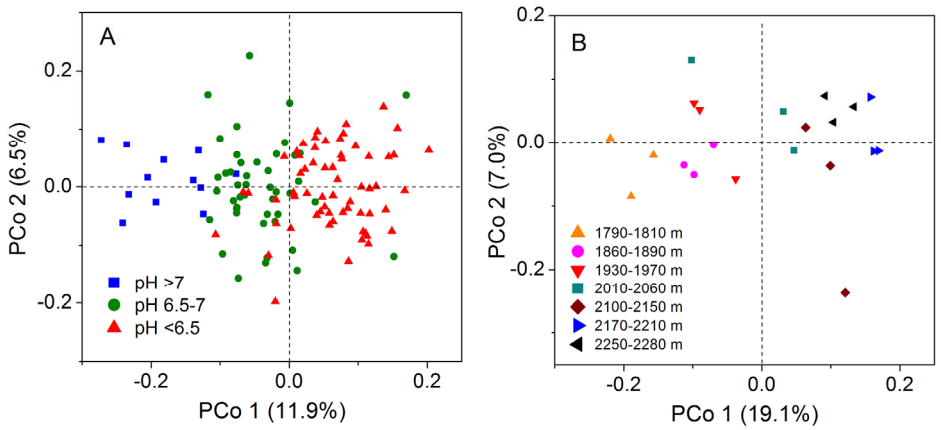

Figure 5 (Color online) PCoA analysis with Bray-Curtis distance matrix of the bacterial communities across pH gradients in the forest (A) and elevation gradients in the meadow (B).

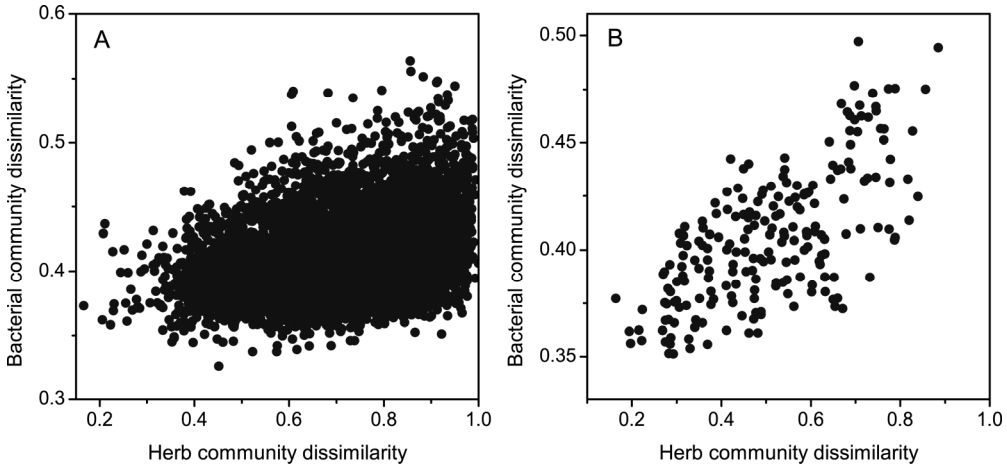

Figure 6 Relationships between herb and bacterial community dissimilarities in the forest (A, R=0.27; P=0.0001) and the meadow (B, R=0.53; P=0.0001). The R value was determined after controlling for total environmental distance and spatial distance. Each point represents the dissimilarity of taxonomic composition between a pair of plots.

(Urbanová et al., 2015). Therefore, changes in plant community composition across the treeline should be paralleled by concomitant changes in soil microbial communities. In this study, relative abundance of dominant phyla and bacterial community structure of the meadow were significantly different from the forest and this result was consistent with another study which reported distinct bacterial communities between coniferous forest and shrubland across the timberline (Ding et al., 2015). This study also found significant positive relationships between the pair-wise dissimilarity of bacterial and herb community composition, despite the limited correlation with high dissimilarity in plant composition among some sites in the forest. With the upshift of the treeline and changes of plant taxonomic composition as a consequence of global climate change (Harsch et al., 2009), profiling microbial communities across different vegetation composition is crucial to predict the dynamic of microbial distribution due to the closely linkages between above- and below-ground organisms (Wardle et al., 2004).

However, the pair-wise dissimilarity of bacterial community was much weaker than that of plant community and showed no spatial effect. This is possibly related to the high dispersal ability of microbes (Finlay, 2002; Tedersoo et al., 2014). As a result, microbial communities are not constrained by spatial distance at the local scale, that is to say, soils sampled from distant locations may not necessarily have distinct microbial communities from those collected in close proximity. Wang et al. also found that microbial communities were varied less across the spatial distance along elevational gradients (Wang et al., 2012b). Even at the continental scale, Fierer and Jackson found that environmental factors were more important in driving the distribution of microbial communities rather than geographic distance (Fierer and Jackson, 2006). Additional studies have also suggested that microbial communities are more confined to local environmental factors (Dequiedt et al., 2009; Shen et al., 2014).

Soil environmental factors played important roles in con-

structing bacterial communities in our study, such as soil pH and temperature, soil available phosphorus, C∶N, and clay %. However, the primary driving factor changed across the treeline. The main factor in controlling soil community structure in the forest was soil pH, which has been well demonstrated in many previous studies (Fierer and Jackson, 2006; Griffiths et al., 2011; Lauber et al., 2009). Soil pH may impose a direct physiological constraint on soil bacteria (Lauber et al., 2009), changing bacterial community structures by altering the relative abundance of dominant bacterial phyla under different pH gradients (Liu et al., 2014; Shen et al., 2013). In this study, *Proteobacteria* was the most dominant phylum rather than *Acidobacteria* in other similar studies (Singh et al., 2012; Wang et al., 2015a). The proportions of *Acidobacteria* (16.5% and 13.1%, respectively) were much lower compared to the 37.8% in Mt. Shegyla on the Tibetan Plateau (Wang et al., 2015a) and 32.5% on Mt. Halla (Singh et al., 2012). This kind of divergence may be explained by the relatively high pH in our study sites, considering the negative correlation shown between *Acidobacteria* and pH found in previous studies (Bryant et al., 2008; Shen et al., 2013). As opposed to the importance of pH in the forest, we found a primary correlation between bacterial community structure and soil temperature in the meadow. Above the treeline, soil temperature dramatically declines along the elevation accompanied by the change of plant community, thus lead to the significant change of soil bacterial community with elevation. Ding et al. also demonstrated soil temperature as the main factor influencing microbial composition around the timberline (Ding et al., 2015). Total variation explained by soil temperature combined with available phosphorus, pH, and clay content reached 67% in the meadow, implying niche-based environmental filtering processes strongly structuring bacterial communities along the elevational gradient (Shen et al., 2015). However, the explanatory power of bacterial diversity in the forest is much lower than that in the meadow. A possible reason is that the soil conditions are more complex due to the litters produced by woody plants in the forest.

In conclusion, along our elevational transect, soil bacterial distribution pattern shifted at the treeline responding to changes in soil environmental factors regulated by aboveground vegetation. Soil temperature, available phosphorus, and pH were more important than other environmental variables in shaping the bacterial community structure and diversity pattern. Bacterial and herb species richness were uncoupled, but bacterial community structure could be predicted by herb community composition. This study stress high spatial resolution and aboveground vegetation matter in detecting microbial patterns along elevational gradients. To better understand how biodiversity and the functioning of entire ecosystem respond to global climate change, more studies must focus on the distribution of soil microbes across the treeline in view of the ecological linkages between below- and above-ground organisms.

MATERIALS AND METHODS

Study site and sampling

Dongling Mountain is located in the Beijing Forest Ecosystem Research Station (30°57′29 N, 115°25′33 E), about 100 kilometers northwest of Beijing, China. This area has a warm temperate continental monsoon climate with average annual precipitation between 500–650 mm and average annual temperature between 5°C–10°C. The forest is a secondary forest around 80 years which is dominated by oak trees (*Quercus liaotungensis*) with a few birches (*Betula* spp.), maples (*Acer mono.*), shrubs (e.g., *Prunus* spp., *Vitex negundo* var. *hetertophylla*), and rich herbaceous plants. The treeline occurs at about 1,770 m, whereupon a meadow extends from the treeline to the mountain's summit (2,300 m).

Soil samples were collected in August 2013, following an elevational gradient from 1,020–2,280 m along the western slope. Below the treeline, 10 transects were set up from 1,020–1,770 m, which constituted a continuous elevational gradient with a width of 10 m. These 10 transects were in turn divided into 119 individual plots (each unit 10 m×10 m). Above the treeline, we established 21 plots (10 m×10 m greater than 50 m apart) along the elevational gradient. Within each 10 m×10 m plot, three herbaceous quadrats (1 m×1 m) were established, recording the abundance and names for all herb species. Soil temperature (ST) was measured by a thermometer with an average of six measurements per plot. Soil samples were then collected below the litter layer at a depth of 10 cm, with a total of six samples from the herb survey quadrats combined together as a single bulk sample in each 10 m×10 m plot. The fresh soil samples were thoroughly mixed, sieved through a 2 mm sieve and divided into two sub-samples. One sub-sample was air-dried for the physical and chemical analyses, and the other was kept at −80°C for DNA extraction.

Soil characteristics analyses

Soil pH was determined at a ratio of 1∶2.5 (soil to water, w/v). Soil moisture (SM) was measured gravimetrically. Soil organic carbon (SOC) was determined by $K_2Cr_2O_7$ oxidation method (Walkley, 1947). Total N and total C were measured by direct combustion using a C/N analyzer (Vario EL III, Germany), and soil C∶N ratio was calculated based on total C and total N. Available phosphorus (AP) was measured using the Mo-Sb anti-spectrophotometry method after extracted. Available nitrogen (AN) was measured through the alkaline hydrolysis diffusion method (Cornfield, 1960). Soil texture was analyzed using a Mastersizer 2000 Laser Diffraction Particle Analyzer (Malvern Instruments, UK). The soil particle size was partitioned into clay (0–2 μm), silt (2–50 μm) and sand (50–2,000 μm) according

to the classification system of the US Department of Agriculture.

DNA extraction

Soil DNA was extracted from 0.25 g of freeze-dried soil using a MOBIO Power Soil DNA extraction kit (MO Bio Laboratories, USA) according to the manufacturer's instructions. DNA quality was assessed by the ratios of 260 nm/280 nm and 260 nm/230 nm, and final DNA contents were quantified using NanoDrop (Thermo Fisher Scientific, USA). All extracted DNA was stored at −80°C until use.

Illumina sequencing

16s rRNA genes was amplified targeting V4-V5 region using primer pair 515F/907R (Forward primer, 515F, 5′-GTGCCAGCMGCCGCGGTAA-3′; reverse primer, 907R, 5′-GGACTACHVGGGTWTCTAAT-3′) combined with adapter sequences and barcode sequences. The targeted gene region has been considered appropriate for an accurate phylogenetic reconstruction of bacteria (Biddle et al., 2008; Shen et al., 2013) and avoiding overestimation (Sun et al., 2013). PCR was conducted at a total volume of 25 μL, containing 4 μL 5× FastPfu Buffer, 2 μL 2.5 mmol L^{-1} dNTPs, 0.4 μL of each primer (5 mmol L^{-1}), 0.4 μL FastPfu Polymerase (TransGen, Beijing) and 10 ng template DNA. The following cycling parameters were used: 95°C for 2 min; 30 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 45 s; followed by 72°C for 10 min. The PCR reactions were performed in triplicate for each sample to minimize potential biases from amplification and then pooled together. The PCR products were purified using the AxyPrepDNA Gel Extraction Kit (AXYGEN, USA). Equal concentrated amplicons were paired-end sequenced (PE 2×300) on Illumina MiSeq platform (Illumina, USA).

Sequences analyses

Quality trimming was done using Trimmomatic (Bolger et al., 2014). Pairs of reads were merged by FLASH (Magoč and Salzberg, 2011) according to the overlap. Primary qualified and merged sequences were analyzed in QIIME v.1.8.0 software package (Caporaso et al., 2010b). Sequencing reads were assigned to each sample according to the unique barcodes of each sample. Sequences were removed if they were shorter than 200 bp, had quality scores <30 in a window of 50 nt, contained ambiguous bases, had more than one mismatches in the primer, or that could not be assigned to a sample using the barcodes. Chimeras were checked using UCHIME (Edgar et al., 2011) against the "RDP Gold" database and were discarded. Operational taxonomic units (OTUs) were picked by pick_otu.py script based on ⩾97% similarity with the USEARCH option (Edgar, 2010). Representative sequences were chosen with the most abundant sequences, and then reference sequences of OTUs were assigned taxonomies using the rdp classifier method against the 13_8 Greegenes database (McDonald et al., 2012). Sequences were aligned to the Greengenes reference alignment using PyNAST (Caporaso et al., 2010a), and a phylogenetic tree was constructed with FastTree according to the standard procedures within QIIME. Sequences failing to aligned, non-bacteria, and rare OTUs (sequences <5) were filtered for the final OTUs table. Alpha and beta diversity were calculated by a randomly selected subset of 11,186 sequences per soil sample in view of the unevenly sequences bias. Representative sequences data have been submitted to GenBank databases under accession numbers from KU147498 to KU156629.

Statistical analyses

Bacterial phylotype richness (OTUs) and phylogenetic diversity (Faith, 1992) were calculated using QIIME to assess the bacterial alpha diversity, and were fitted against elevation in quadratic and linear regression in both the forest and grassland. Goodness of fit was evaluated by adjusted R^2 and P value.

The relationship between bacterial alpha diversity and potential explanatory variables were assessed using multiple ordinary least squares (OLS) regression. All environmental variables and biodiversity metrics were standardized at a mean of 0 and a standard deviation of 1. The best models were identified according to Akaike's information criterion (AIC) (Akaike, 1987). Spatial autocorrelation was considered (not in the meadow) by including eigenvector-based spatial filters derived from geographic distances in all regression models (Diniz-Filho and Bini, 2005; Wang et al., 2011). Before applying OLS, variable clustering was used to assess the redundancy of the environmental variables (Harrell, 2001). Using the VARCLUS procedure in the Hmisc R package, we removed the sand%, elevation, AN, TN, TC (Spearman ρ^2>0.46) from the following analysis both in the forest and meadow. The multiple regressions considered spatial autocorrelation analysis were performed in the SAM 4.0 software (Rangel et al., 2010).

PCoA analysis based on Bray-Curtis dissimilarity matrices was performed to examine the bacterial community variation and a permutational matrix-based multivariate analysis of variance (Adonis test, Vegan package in R) was used to test the significance. A partial mantel test in the ecodist R package (Goslee and Urban, 2007) was used to investigate the relationship between bacterial community dissimilarity and plant community dissimilarity, environmental distance, and geographic distance (Martiny et al., 2011). Bray-Curtis dissimilarity matrices of bacterial and plant community were generated based on the Hellinger-transformed abundance data (Prober et al., 2015) as well as the Euclidean distances of environmental variables (standardized) and geographic distance. Geographic distance was applied in the forest analysis (not in the meadow) to estimate the effect of spatial autocorrelation. To tease apart the relative importance of the environmental variables, multiple

regression on distance matrices (MRM, an extension of partial Mantel analysis) models (Lichstein, 2007) were applied. The best models were determined using the backward elimination to select the potential explanatory variables (P<0.05). All the models were performed with 9999 permutations and analyzed in R statistical software (R Development Core Team 2013).

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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SUPPORTING INFORMATION

Figure S1 Herb species richness across the elevational gradients at Dongling Mt. China. The dotted line represents the occurrence of treeline.

Figure S2 Relationships between herb species richness and bacterial species richness.

Table S1 Partial Mantel tests for the correlation of bacterial community dissimilarity with environmental distance, spatial distance and plant community dissimilarity using Pearson's correlation. Explanatory distance was analysed after controlling other distance matrices

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